Presence and Persistence of Zika Virus RNA in Semen, United Kingdom, 2016

Technical Appendix

Technical Appendix Table 1. Characteristics of patients with real-time RT-PCR-confirmed Zika virus infection who were evaluated

to determine presence and persistence of Zika virus RNA in semen samples, United Kingdom, 2016*

10 4010	line presence a	oresence and persistence of Zika virus RNA in semen samp Initial Zika virus diagnostic sample In				Initial flavivirus serostatus			First semen sample	
Travel		Day Sample		Zika virus	Zika virus	Dengue Previous		Zika virus		
Patient	history	PSÓ	type	test result	antibodies†	IgЇ	dengue	Day PSO	RNA result	
1	Cook	Day 3	Serum	RNA	Not tested	IND	ND	Day 27	Detected	
	Islands	-	(No urine)	detected				-	Ct 29	
	Feb 2014			Ct 35						
2	Colombia	Day 1	Serum	RNA	IgM not detected	Not	No	Day 26	Detected	
	Dec 2015		(No urine)	detected	IgG not detected	detected			Ct 27.1	
				Ct 37.6						
3	Colombia	Day 3	Serum	RNA	IgM not detected	Detected	Yes	Day 23	Not	
	Dec 2015		(No urine)	detected	IgG not detected				detected	
				Ct 37.3		• • •				
4	Venezuela	Day 1	Urine§	RNA	IgM not detected	Not	No		Not	
	Jan 2016			detected	IgG not detected	detected		Day 10	detected	
_				Ct 30.7	1.14 . 1				D	
5	D	D0	L lodge of C	RNA	IgM not detected	Not	No	D 04	Detected	
	Brazil	Day 3	Urine§	detected	IgG not detected	detected		Day 21	Ct 21.3	
	Feb 2016			Ct 34.3						
6	Barbados		Plasma	RNA	IgM not detected	Detected	Yes		IND (sub-	
O	Mar 2016	Day 5	(No urine)	detected	IgG not detected	Detected	165	Day 16	threshold	
	Ivial 2010	Day 3	(No dilite)	Ct 32.9	igo noi detected			Day 10	only)¶	
7	Guadeloupe		Plasma	RNA	IgM not detected	Not	No	Day 13	Detected	
•	Apr 2016	Day 2	(No urine)	detected	IgG not detected	detected	140	Day 10	Ct 24.0	
	7.01 2010	Day L	(110 011110)	Ct 30.1	igo not dotoctod	doloolod			0121.0	
8	Brazil	Day 1	Plasma	RNA	IgM not detected	Not	No	Day 13	Detected	
	Apr 2016	,	(No urine)	detected	IgG not detected	detected			Ct 19.2	
	'		,	Ct 39.6	o .					
9	Brazil	Day 2	Plasma	RNA	IgM not detected	Not	No		Detected	
	May 2016	-	(No urine)	detected	IgG not detected	detected		Day 9	Ct 27.5	
				Ct 37.8						
10	Dominican		Serum &	RNA	IgM not detected	Detected	Yes		Detected Ct	
	Republic	Day 1	urine	detected	IgG not detected			Day 14	21.6	
	Jun 2016			Ct 37.2 in						
				serum						
				Ct 30.4 in						
44	Oner	D-::0	I India - C	urine	InM Detected	NI-1	N1-	Day: 00	NI-1	
11	Grenada	Day 3	Urine§	RNA	IgM Detected	Not	No	Day 22	Not	
	Jun 2016			detected	IgG not detected	detected			detected	
12	Grenada	Day 4	Urine§	Ct 31.1 RNA	IgM INDET	Not	No	Day 12	Not	
12	Jun 2016	Day 4	Offices	detected	IgW INDE	detected	INO	Day 12	detected	
	Juli 2010			Ct 30.8	igo noi detected	uelecieu			uelecteu	
13	Jamaica	Day 3	Plasma	RNA	IgM not detected	Not	No	Day 50	Detected	
10	Jun 2016	Day 3	(No urine)	detected	IgG not detected	detected	140	Day 30	Ct 29.5	
	0di1 2010		(140 dililo)	Ct 32.4	igo noi acicolea	doloolod			01 20.0	
14	Mexico	Day 6	Urine§	RNA	IgM Detected	Not	No	Day 63	Detected	
	Jul 2016	Day 0	Oilliog	detected	IgG not detected	detected	140	Day 00	Ct 37.7	
	30. 20.3			Ct 26.9	.3	30.00.00			0. 5	
15	Mexico	Day 2	Plasma	RNA	IgM not detected	Not	No	Day 10	Not	
	Jul-Aug	, -	(No urine)	detected	IgG not detected	detected			detected	
	2016		` ,	Ct 37.7						

		Initial Zika virus diagnostic sample			Initial flav	First semen sample			
	Travel	Day	Sample	Zika virus	Zika virus	Dengue	Previous		Zika virus
Patient	history	PSO	type	test result	antibodies†	IgG‡	dengue	Day PSO	RNA result
16	Barbados	Day 3	Serum	RNA	IgM not detected	Not	No	Day 38	Detected
	Aug 2016	-	(No urine)	detected	IgG not detected	detected			Ct 31.8
	_			Ct 33.8					

^{*}Ct, cycle threshold; IND, indeterminate; ND, not determined; PSO, post symptom onset. †Euroimmun Anti-Zika Virus ELISA (IgM) and Euroimmun Anti-Zika Virus ELISA (IgG). ‡Panbio dengue IgM capture ELISA and dengue IgG indirect ELISA.

Technical Appendix Table 2. Characteristics of patients with serologically diagnosed Zika virus infection who were evaluated to determine presence and persistence of 7ika virus RNA in seman samples. United Kingdom, 2016*

determin	termine presence and persistence of Zika virus RNA in semen samples, United Kingdom, 2016*								
		Initial Zika virus diagnostic sample			Other flavivii	us serology†	First semen sample		
	Travel		Sample	Zika virus test			Day	Zika virus	
Patient	history	Day PSO	types	result‡	Dengue§	Yellow fever¶	PSO	RNA result	
17	Brazil	Day 11	Serum	IgM detected	IgG detected	IgG detected	Day 34	Not detected	
	Feb 2016		Plasma	IgG detected	IgM not detected	(IgM not tested)			
			(No urine)	RNA not detected	RNA not	(RNA not tested)			
			_		detected				
18	Colombia	Day 10	Serum	IgM detected	IgG detected	IgG detected			
	Dec 2015		Plasma	IgG detected	IgM not detected	(IgM not tested)	Day 10	Not detected	
			Urine	RNA not detected	RNA not	(RNA not tested)			
10		D 10		1.14	detected	10111	D 04	5	
19	Guyana	Day 12	Serum	IgM not detected	IgG detected	IgG detected	Day 31	Detected	
	Apr 2016		(No urine)	IgG detected#	IgM not detected	(IgM not tested)		C _t 29.4	
				RNA not detected	RNA not	(RNA not tested)			
20	Ctlusia	Doy 7	Corum	IaM datastad	detected	IaC datastad	Dov. 16	Not detected	
20	St Lucia Jun 2016	Day 7	Serum Plasma	IgM detected IgG detected	IgG detected IgM not detected	IgG detected (IgM not tested)	Day 16	Not detected	
	Juli 2016		(No urine)	RNA not detected	RNA not	(RNA not tested)			
			(INO dilile)	INIA Hot detected	detected	(INIVATIOLIESIEU)			
21	St Vincent	Day 6**	Serum	IgM detected	IgG not detected	IgG not detected	Day 14	Not detected	
	Jul 2016		Plasma	IgG not detected	IgM not detected	(IgM not tested)	,		
			(No urine)	RNA not detected	RNA not	(RNA not tested)			
			,		detected	,			
22	Barbados	Day 16	Serum	IgM detected	IgG not detected	IgG detected	Day 28	Detected	
	Jul 2016		(No urine)	IgG detected	IgM not detected	(IgM not tested)		Ct 24.0	
				RNA not detected	RNA not	(RNA not tested)			
					detected				
23	Jamaica	Day 16	Serum	IgM detected	IgG detected	IgG detected	Day 28	Not detected	
	Jul-Aug		(No urine)	IgG detected	IgM not detected	(IgM not tested)			
	2016			RNA not detected	RNA not	(RNA not tested)			
					detected				
*C _t , cycle threshold; IFA, immunofluorescence assay; PSO, post symptom onset.									

[§]No Zika virus RNA was detected in the accompanying serum/plasma sample.

Ct of 40 was the set diagnostic cut-off; however this sample produced a subthreshold curve, as did the contemporary urine. Of note, this individual was reported to have previously had a vasectomy.

[†]All samples also tested for chikungunya virus, no positives detected.

[‡]Euroimmun Anti-Zika Virus ELISA (IgM) and Euroimmun Anti-Zika Virus ELISA (IgG). §Panbio dengue IgM capture ELISA and dengue IgG indirect ELISA.

[¶]Euroimmun yellow fever IgG IFA.

[#]This serological pattern (a strongly positive Zika IgG and undetectable Zika IgM) is commonly observed in individuals who have previously had dengue virus infection.

**This patient seroconverted; Zika IgM and IgG antibodies were undetectable in a sample taken on Day 4, but IgM was strongly

positive on Day 6 and Day 14, and IgG became weakly positive on Day 14.